Fluorescence-based methods to study hyperthermophilic archaeal family B DNA polymerase: Measurements of DNA binding and real-time enzymatic activities

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We report fluorescence-based methods to study polymerase and exonuclease activities of thermostable DNA polymerases in real-time on primer/template DNA duplex (P/T).

The methods are based on the use of (*i*) a fluorescent reporter which becomes fluorescent upon binding to newly synthesized double-stranded DNA, (*ii*) a quencher reporter from a tripartite Primers/Template (P/T) DNA duplex in strand-displacement polymerase assays, (*iii*) fluorescence fading of a nucleic acid stain concomitantly to DNA degradation in exonuclease assays, (*iv*) steady-state anisotropy to evaluate polymerase-P/T DNA duplex binding affinities. These methods are compatible with standard spectrofluorimeters, plate-readers or real-time PCR instruments.

Here, the efficiency of primer extension and degradation on P/T DNA duplex by *Pyrococcus abyssi* thermostable family B DNA polymerases (PabPolB) has been monitored at 55°C in real-time. In addition, the size of extension products was systematically examined by gel electrophoresis followed by fluorescence visualization[1]. Besides, The equilibrium apparent dissociation constant (K_D) characterizing the PabPolB-P/T DNA complex was calculated by fitting the plot of steady-state anisotropy versus PabPolB concentrations with a Hill model[2].

These real-time methods are very sensitive, quantitative, and well suited for the screening of DNA synthesis and degradation activities by different DNA polymerases. As such, novel intrinsic properties might be discovered with possible evolutionary scenario for the origin of families DNA polymerases. More generally, these fluorescent assays might be applied to other nucleic acid enzymes like helicases or nucleases.

Bibliography

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